TRANSMISSION OF EPIDEMIC INFLUENZA VIRUS IN MICE BY CONTACT

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The susceptibility of mice to the virus of human epidemic influenza and the virus of swine influenza was demonstrated by Andrewes, Laidlaw, and Smith (1934) and Francis (1934) shortly after the virus was first transmitted from human beings to ferrets by Smith, Andrewes, and Laidlaw in 1933. In ferrets, intranasal instillation of influenza virus produces fever, symptoms of nasal involvement, and sometimes consolidation of one or more lobes of the lungs. The nasal lesions in the ferret are essentially a necrosis and desquamation of the respiratory epithelium, as shown by Francis and Stuart-Harris (1938). Normal ferrets placed in the same cage with infected animals contract the disease and show not only the temperature response and symptoms characteristic of influenza, but also typical influenzal lung lesions (Smith, Andrewes, and Stuart-Harris, 1938).

Intranasal instillation of influenza virus into mice produces a disease which is essentially a virus pneumonia. There are no symptoms of nasal involvement with the possible exception of sneezing. Andrewes, Laidlaw, and Smith (1934) reported that in one experiment a group of mice exposed to others infected with swine influenza virus apparently acquired an immunity without having shown symptoms of the disease, but this result was not regularly reproducible. In a later report Andrewes, Smith, and Stuart-Harris (1938) state that influenza virus in mice is not transmissible by contact but only by direct inoculation of virus into the respiratory tract under anesthesia. Francis, working in this laboratory in 1937 and using the PR8 strain of virus,

obtained rather irregular contact infections in mice without lung lesions.[1]

This paper will report the results of contact experiments in which a large percentage of groups of normal mice became infected with influenza virus and developed moderate to extensive consolidation of the lungs by contact with mice infected with human or swine strains. Infection of unanesthetized mice by ingestion of virus has also been found to occur, but this is much less regular than infection by contact.

METHOD

Mice were kept in glass jars 22 cm. high and 16 cm. in diameter with weighted wire mesh lids. Bedding consisted of a layer of pine shavings. Throughout this work the method of performing the contact experiments was always the same. Eight mice were inoculated intranasally under light ether anesthesia with a 10 per cent suspension of infected mouse lung or dilution of this suspension. The eight inoculated mice were then put into a jar for 24 hours or overnight. At the end of this time the inoculated mice were marked with dye and placed in a new jar with 8 normal mice, care being taken to avoid transferring shavings or bread from the original infected jar. In this paper the mice given virus intranasally will be designated as "inoculated mice" and the normal mice placed in contact in the same jar with these inoculated mice will be designated as "contact mice."

Between 3 and 7 days, depending upon the amount of virus given, the inoculated mice died and were removed from the jar as soon as possible after death in order to prevent their being eaten by the contact mice. At the end of 10 to 11 days the contact mice and any surviving inoculated mice were killed. The lungs of the contact mice were removed aseptically and examined for lesions. Lesions were recorded as +, ++, +++, and ++++ according to the proportion of the volume of the lungs showing consolidation. The lungs of the entire contact group were combined, ground with alundum, and made into a 10 per cent suspension in broth. This suspension was then

[1] T. Francis, Jr.: Personal communication.

inoculated intranasally into eight mice, and the contact experiment repeated as described above.

It has not yet been possible to obtain consistent transmission of virus by multiple contact passages without intervening intranasal inoculation of mice. Even when alternate passages by contact and intranasal inoculation are performed, the virus may die out after several passages.

When the lungs of the contact mice were found to be negative, at least two more passages by intranasal inoculation were made before the absence of virus was considered probable. In certain instances "inapparent" infections resulting from contact were detected in this way.

LUNG LESIONS PRODUCED IN THE CONTACT MICE

When the contact mice were killed at the end of 10 to 11 days, the lungs of a certain percentage of them showed large or small lesions typical of influenza. The consolidated portion of the lung was a solid red color, generally having a tinge of purple, and these lesions did not differ in appearance from the lesions found in the lungs of mice 10 days after intranasal inoculation, except that sometimes they appeared to be slightly lighter in color.

Death of the contact mice occurred irregularly and infrequently. About two out of each hundred contact mice studied have died before the end of the 11-day period with almost complete consolidation of the lungs. Experiments described in a later section of this paper indicate that the amount of virus in the lungs of the contact mice decreases quite rapidly after 10 days.

DIFFERENCES IN CONTACT TRANSMISSION OF STRAINS OF INFLUENZA VIRUS

The results of several serial passages alternately by contact and intranasal instillation of four different strains of influenza virus are presented in table 1. The PR8 strain was isolated by Francis in 1934 and has been through about 350 passages in mice. The W.S. strain was the first strain of influenza virus to be isolated by Wilson Smith, Andrewes, and Laidlaw in 1933 and has been through about 400 mouse passages. The Mel-

bourne strain[2] has been kept in tissue culture since first isolated and has been through only two or three animal passages. The swine strain has been through 40 mouse passages since it was isolated from swine.

The second column of the table shows the number of series and the number of contact passages in each series done with the various strains of influenza virus. With PR8 there were three series consisting of four, five, and six passages by contact, alternating with an equal number of passages by intranasal inoculation. In the first series the virus died out after four contact passages. This was apparently due to the fact that the inoculated mice in the last two passages received a 0.1 per cent sus-

TABLE 1

Differences in transmission by contact of four strains of influenza virus

STRAIN OF INFLUENZA VIRUS	TOTAL* NUMBER OF SERIAL PASSAGES BY CONTACT	RANGE OF PER CENT OF CONTACT MICE HAVING LESIONS	AVERAGE PER CENT OF CONTACT MICE HAVING LESIONS
PR8	4, 5, 6	12–100	62
W.S.	12	12–100	73
Melbourne	1, 4	0–50	25
Swine	1, 0, 3	0–37	11

* Eight mice used in each passage. Alternate passages by contact and intranasal inoculation. Number of contact passages only, for each series, is given in table.

pension of mouse lung instead of the usual 10 per cent suspension. In the two other series of five and six passages with PR8 in 10 per cent suspension of mouse lung there was no diminution of virus activity. The W.S. strain has been carried in one series of twelve passages by alternate contact and intranasal inoculation and can apparently be carried indefinitely in this way.

In the first series with the Melbourne strain the virus was transmitted in the first passage by contact but was not transmitted in the second contact experiment of the series. In the second series this strain was successfully carried through four passages by contact, but in the last three passages only one out

[2] Obtained from Dr. F. M. Burnett in 1937.

of the eight mice in each group showed lesions in the lungs. The series was discontinued at this point. In the first experiment with the swine influenza virus no lesions appeared in the lungs of the contact mice, but subinoculation of these lungs showed the presence of virus. In a second experiment the swine virus was not transmitted by contact, and in the third series it was carried for three passages and then died out.

The figures in the third and fourth columns of table 1 represent the percentages of each of the groups of contact mice in which consolidation in the lungs was found. With the PR8 and W.S. strains, from one to eight of the contact mice in each group showed lesions, and large and small lesions were about equally frequent. With the PR8 strain 62 per cent of 120 contact mice had lung lesions, while with the W.S. strain 73 per cent of 96 contact mice had lesions. With the Melbourne and swine strains the percentages were considerably lower.

These results indicate that the PR8 and W.S. strains, which have been through many passages in mice, are more easily tranmissible by contact than the Melbourne and swine strains, which have been through relatively fewer passages. These differences cannot be directly correlated with virulence for mice by intranasal inoculation because the latter two strains kill mice at approximately the same dilutions as the PR8 and W.S. strains. The PR8 and W.S. strains passed by contact have been identified as influenza virus by neutralization tests with human serum from patients convalescent from influenza.

EFFECT OF TIME OF EXPOSURE OF CONTACT MICE TO INOCULATED MICE

In one series of experiments with the PR8 strain of influenza virus the inoculated mice were placed in the jars with the contact mice 24 hours after inoculation, and the eight contact mice were then removed from each jar after additional periods of 24 to 96 hours and placed in clean jars. Similar experiments were performed with the W.S. strain. In another experiment the inoculated mice were placed with the contact mice after periods of time up to 4 days after inoculation, and the contact mice were

allowed to remain in the same jar until the conclusion of the experiment.

The results are presented in table 2. In the experiments with the PR8 strain it appeared that 24 to 48 hours of contact, either between the 1st and 2nd day after inoculation, or from the 4th day to the death of all of the inoculated mice, was insufficient to permit transmission of the virus to the contact mice. Infection of a significant percentage of the contact mice occurred when they

TABLE 2

Effect of time of contact on transmission of influenza virus

STRAIN OF VIRUS	DAY IN CONTACT AFTER INOCULATION	TOTAL TIME OF CONTACT	AVERAGE LIFE OF INOCULATED MICE	NUMBER OF EXPERIMENTS	NUMBER OF CONTACT MICE	LUNG LESIONS IN CONTACT MICE
		hours	*days*			*per cent*
PR8	1	24	4.6†	3	16	0
	1	48	4.7†	3	16	12
	1	72	4.3†	2	12	33
	1	96	4.4	3	16	58
	1	103*	5.3	2	14	50
	2	82*	5.4	2	14	43
	3	72*	6.0	2	10	20
	4	34*	5.4	1	8	0
W.S.	0	48	4.6†	5	40	60
	1	24	4.9†	1	4	50
	1	48	4.9†	1	4	50
	1	72	5.8	1	4	100
	1	96	5.8	1	4	100

* Based on average life of inoculated mice shown in next column.

† In these experiments no mice died before contacts were removed from jar.

were placed with the inoculated mice on the 1st or 2nd day and allowed to remain for 72 hours or more. When the mice were placed in contact on the 3rd day after inoculation, only 20 per cent of the contact mice became infected.

The W.S. strain infected a higher percentage of mice during a period of contact of 24 to 48 hours than did the PR8 strain. This is indicated by the figures in the lower part of table 2.

In several of the experiments in this series, infection of the contact mice had occurred when they were removed from the jar

at a time when all of the inoculated mice were still living. This excludes the possibility that these mice became infected by eating parts of the inoculated mice. Also in the three experiments in which the mice were placed in contact shortly before the death of the inoculated mice the amount of infection by contact was small despite the opportunities for cannibalism. Direct experiments on ingestion of virus will be presented in a later section.

EFFECT OF AMOUNT OF VIRUS GIVEN TO INOCULATED MICE ON TRANSMISSION BY CONTACT

Groups of eight mice were inoculated intranasally with various dilutions of influenza virus containing a known number of minimal lethal doses from 1:10 ot 100,000.[3] The dilution containing

TABLE 3

Effect of amount of virus in inoculated mice on transmission of PR8 strain by contact

M.L.D. TO INOCULATED MICE	NUMBER OF EXPERIMENTS	NUMBER OF CONTACT MICE	MICE WITH LARGE LESIONS	MICE WITH SMALL LESIONS	MICE WITH NO LESIONS
			per cent	*per cent*	*per cent*
1:10	1	8	0	25	75
1	1	8	0	25	75
10	1	8	0	25	75
100	4	31	12	12	76
1,000	4	32	12	48	40
10,000	5	36	55	33	12
100,000	3	24	33	42	25

1:10 M.L.D. represented approximately 10 minimal infectious doses so that lesions were produced in the lungs of all of the mice receiving this dose of virus, but the animals did not die. The inoculated mice were then placed in contact with eight normal mice for a period of 10 days or until the death of the inoculated mice.

The results of experiments with the PR8 strain are summarized in table 3. Small lesions were found in the lungs of some of the mice which had been in contact with inoculated mice receiving as little as 1:10 to 1 M.L.D. of virus, but progressively larger

[3] The minimal lethal dose is defined as the least amount of a suspension of virus that will kill 50 per cent of the inoculated mice in 10 days or less.

and more frequent lesions were found in the contact mice as the dose given to the inoculated mice was increased up to 10,000 M.L.D. Most of the mice receiving 100,000 M.L.D. died on the 3rd or 4th day; those receiving 1000 and 10,000 M.L.D. died on the 4th to 6th day, while those receiving smaller doses died on the 6th to 9th day. Thus, the contact mice were exposed for a longer time to the infected mice which received the smaller doses of virus, and this should favor transmission of the virus. If the periods of exposure were made uniform throughout, it would appear that less transmission by contact would have occurred at the higher dilutions of virus.

TABLE 4

Effect of amount of virus in inoculated mice on transmission of W.S. strain by contact

M.L.D. TO INOCULATED MICE	NUMBER OF EXPERIMENTS	NUMBER OF CONTACT MICE	MICE WITH LESIONS	MICE WITH NO LESIONS
			per cent	*per cent*
1:10	3	23	0	100
1	3	23	9	91
10	2	14	35	65
100	2	15	33	67
1,000	3	23	43	57
10,000	6	45	78	21

The results of similar experiments with the W.S. strain are summarized in table 4. In these experiments no infections occurred in the mice placed in contact with inoculated mice receiving 1:10 M.L.D. of virus. In two out of three experiments in which the inoculated mice received 1 M.L.D., one mouse out of each group of eight contacts developed lesions. With larger amounts of virus the results were similar to those obtained with the PR8 strain.

EFFECT OF AGE OF THE MICE

Contact experiments were done using mice from 16 days to 8 weeks of age in groups of sixteen (8 inoculated mice and 8 contact mice). The results are presented in table 5. From a consideration of the percentages given in the fifth and last columns of the table it appears that in the experiments with both the

PR8 and W.S. strains the youngest contact mice had fewer extensive lesions in their lungs and a larger percentage showed no lesions than did the mice of 4 and 8 weeks of age. The oldest inoculated mice lived, on the average, about 12 hours longer than the youngest. The differences cannot be attributed to a longer period of contact when older mice were used, but the greater degree of infection of the older contact mice may have been due to more intimate contact. Mice 8 weeks of age being about twice as large as those 16 days old, it is obvious that more crowding would occur in the jars containing the older mice.

TABLE 5

Effect of age of mice on transmission of influenza virus by contact

STRAIN OF VIRUS	AGE OF MICE	NUMBER OF EXPERIMENTS	TOTAL NUMBER OF CONTACT MICE	MICE WITH LARGE LUNG LESIONS	MICE WITH SMALL LUNG LESIONS	MICE WITH NO LESIONS
				per cent	*per cent*	*per cent*
W.S.	16 days	3	23	22	44	33
	4 weeks	3	23	57	26	17
	8 weeks	2	16	69	25	6
PR8	16 days	2	15	6	47	47
	4 weeks	2	16	44	50	6
	8 weeks	1	7	57	28	15

MICE FROM DIFFERENT BREEDERS

Swiss mice from seven breeders were tested with the PR8 and W.S. strains of virus for their ability to transmit influenza virus by contact. Positive results were obtained with mice from all of the seven sources, and the variations in percentage of contact mice showing lung lesions was not great enough to be considered significant. Only the Swiss strain of mice has been tested.

SURVIVAL OF VIRUS IN THE CONTACT MICE

Three groups of 8 mice infected by contact, using the PR8 strain of virus, were killed at 10,15, and 20 days, respectively, after they had first been placed in jars with inoculated mice. The lungs of each group were made up in a 10 per cent suspension in broth, and this was inoculated intranasally into six normal

mice. The results are presented in table 6. Mice subinoculated from contact mice killed on the 10th day died in 4 to 5 days, indicating the presence of a large amount of virus in the lungs of the contact mice. On the other hand, mice subinoculated on the 15th day from the contacts showed large lesions, but five of the six survived, while of six mice inoculated on the 20th day from the contacts only two had small lung lesions. These results indicate that the virus gradually disappears from the lungs of the contact mice after 10 days. This disappearance of virus apparently coincides with the development of active immunity.

TABLE 6

Survival of virus in contact mice

TIME OF PASSAGE FROM CONTACTS	LESIONS IN LUNGS OF CONTACT MICE	MICE SUBINOCULATED INTRANASALLY WITH LUNGS OF CONTACT MICE*
days		
10	++, ++, +, +, +, 0, 0, 0	4, 4, 4, 5, 5, 10
15	+++, +, +, +, +, +, +, 0	10, +++, +++, +++, ++, ++
20	++, +, +, 0, 0, 0	+, +, 0, 0, 0, 0

* Numbers in this column represent time of death in days after inoculation.

INGESTION AND EXTERNAL APPLICATION OF VIRUS COMPARED WITH INFECTION BY CONTACT

It was apparent that the fur of the inoculated mice became contaminated with a certain amount of virus suspension after intranasal instillation. Despite the fact that the inoculated mice were kept by themselves for 24 hours before being placed in the jar with the contact mice, it seemed possible that some external virus might remain. In several of the experiments the dead inoculated mice were eaten by the contact mice before they could be removed. These facts made it necessary to perform control experiments in which a 10 per cent suspension of infected mouse lung was applied to the fur of normal mice. In other experiments mice which had been given no food or water for 24 hours were fed bread soaked in virus suspension or allowed to eat infected lungs.

The results of these experiments are presented in table 7. For comparison the results of several contact experiments in

which cannibalism was excluded are also included in the table. In three experiments external application of virus suspension to the fur failed to produce infection as determined by the negative results of two successive subinoculations of the lungs of mice treated in this way. Two ingestion experiments with the W.S. strain were likewise negative. In two experiments with the PR8 strain lesions were found in the lungs of some of the mice which had ingested bread soaked in virus suspension. The results indicate that lesions may be produced in the lungs of mice

TABLE 7

Comparison of infection by contact and by ingestion

STRAIN OF VIRUS	MODE OF INFECTION	NUMBER OF EXPERIMENTS	NUMBER OF MICE	PER CENT OF MICE SHOWING LUNG LESIONS
W.S.	Contact with infected mice, no ingestion	3	24	66
	Ingestion of infected lung	1	6	0
	Ingestion of virus suspension on bread	1	6	0
	Virus suspension applied to fur	1	16*	0
PR8	Contact with infected mice, no ingestion	4	32	47
	Ingestion of virus suspension on bread	2	12	37
	Virus suspension applied to fur	2	20	0

* In this experiment virus was applied to the fur of 8 mice and these were placed in a jar with 8 normal mice.

by ingestion of virus-containing material, but this is less regular than infection with the same strains by contact. Contamination of the fur of the inoculated mice after intranasal instillation apparently does not play an important part in transmission of the virus by contact.

DISCUSSION

In view of the failure of some investigators to obtain either lung lesions, or transmission of the virus of epidemic influenza in mice by contact, the positive findings reported in this paper must be attributed to differences in the strains of virus used or to

differences in the experimental conditions. The irregular results obtained with the swine and Melbourne strains, which had been through few mouse passages, compared with the relative ease of contact transmission of the W.S. and PR8 strains, which have been through many mouse passages, suggest that adaptation of the virus to mice may play a part. It remains to be seen whether or not many serial passages alternately by contact and intranasal inoculation will increase the infectivity of the virus for mice to such an extent that the contact mice may contract fatal infections and epidemics may be produced in colonies of mice.

In the experiments reported in this paper the crowding of inoculated and contact mice into one jar seems to have been an important factor. There is no direct evidence that virus may be transmitted from infected mice in one jar to normal mice in an adjacent jar, but the possibility of this happening occasionally with such strains as PR8 and W.S. cannot be disregarded.

The mode of transmission of the virus in mice by contact is not yet known. Because of the negative results of the experiments in which virus suspension was applied to the fur of mice, it seems unlikely that contamination of the jar or its contents plays an important part. Probably the infectious agent passes directly between mice either by droplet infection, as it apparently does in ferrets, or by nasal contact of one animal with another. Mice infected with influenza virus have a bubbling type of respiration in the last stages of the disease so that there would seem to be opportunity for dissemination of infected droplets.

Certain unexplained irregularities in the amount of transmission of the same strain of virus in mice from the same dealer in different experiments performed at various times in the course of this work may be due to factors other than those so far studied. However, the strain of virus, the time of contact, and the concentration of virus in the inoculated mice appear to be the principal factors which influence contact transmission.

SUMMARY

Normal mice placed in jars with mice infected with epidemic influenza virus will under certain conditions contract an infection

which is evident as moderate to extensive consolidation of the lungs but which is seldom fatal.

The PR8 and W.S. strains of human influenza virus are more readily transmitted by contact than are the Melbourne and swine strains.

The degree of infection of the contact mice is related to the concentration of virus given to the inoculated mice and to the time of contact.

External application of virus does not cause demonstrable infection of mice, but ingestion of virus may produce lung lesions in a certain percentage of a group of mice.

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